Virology 256, 371–380 (1999)

Analysis of Human Lymphotropic T-Cell Virus Type II-like Particle Production by Recombinant Baculovirus-Infected Insect Cells

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Received November 12, 1998; returned to author for revision December 8, 1998; accepted February 11, 1999

The molecular processes involved in retrovirus assembly and budding formation remain poorly understood. The *gag-pro-pol* genes of human lymphotropic T-cell virus type II (HTLV-II) are translated into Gag, Gag-Pro, or Gag-Pro-Pol by frameshift events. In the present study, we investigated the roles of the *gag, pro,* and *pol* regions of HTLV-II in viral particle formation using recombinant baculoviruses. In this study we could successfully produce mature HTLV-II viral particles containing core structures using a construct expressing the entire *gag-pro-pol* region. We also investigated the role of the *pol* region in particle formation. Deletion of the *pol* region affects viral particle assembly or release very little, indicating that the *gag-pro* region is sufficient for viral particle formation and maturation. Expression of the Gag proteins alone or Gag proteins with inactivated viral proteases (Pro) resulted in the formation of viral particles; however, these particles did not contain core structures. These results suggest the intracellular expression of Gag with Pro of HTLV-II is essential for the production of mature virus particles, whereas that of Pol is not.

INTRODUCTION

The human lymphotropic T-cell virus type II (HTLV-II) is a retrovirus that is associated with rare hematological and neurological disorders (Hall et al., 1996). Sequence analysis of the proviral DNA of both human T-cell lymphotropic viruses (HTLV), HTLV-I, and HTLV-II, has demonstrated a unique arrangement of the *gag* and *pol* genes (Mador *et al.,* 1989).

The internal core proteins and the enzymes involved in RNA reverse transcription and integration are encoded by the *gag* and *pol* genes, respectively. HTLV-II also has the *pro* gene, which codes for the viral protease (Pro) and is located between the *gag* and *pol* genes (Mador *et al.,* 1989). These proteins are initially translated as Gag, Gag-Pro, and Gag-Pro-Pol polyproteins in a frameshift fashion and then undergo post-translational processing by Pro, producing the individual proteins. The internal core protein Gag of HTLV-II is initially translated as a polyprotein precursor ($Pr53^{gag}$) consisting of 429 amino acid residues; this is also processed by Pro into three mature forms: p19 (the membrane-associated matrix protein, MA), p24 (the capsid protein, CA), and p15 (the nucleocapsid protein, NC) (Shimotohno *et al.,* 1985).

The production of individual HTLV-I Gag proteins or precursor proteins has been demonstrated using various expression systems. The production of p19, p24, and p15 in *Escherichia coli* (Itamura *et al.,* 1985; Sekine *et al.,* 1989) and of $Pr53^{gag}$ in *Saccharomyces cerevisiae* (Hayakawa *et al.,* 1992) has been reported. In addition, the production of HTLV-I viral particles has been described in mammalian cells (Derse *et al.,* 1995; Kimata *et al.,* 1994) but not in the baculovirus system.

Expression of human immunodeficiency virus type 1 (HIV-1) Gag-Pol is known to produce mature viral particles containing processed structural proteins (Ross *et al.*, 1991; Shioda and Shibuta, 1990). In contrast, expression of the Gag precursor protein alone produced morphologically immature particles devoid of electron-dense cores (Gheysen *et al.,* 1989). This observation indicates that maturation of Gag particle requires the coordinated expression of the *pol* gene (Shioda and Shibuta, 1990). The Pol protein of HIV-1 is processed into protease (Pro), reverse transcriptase (RT), and integrase (IN). Mutational analyses in IN and/or RT result in the premature activation of the HIV-1 Pro, which in turn leads to a reduction in particle production (Bukovsky and Göttlinger, 1996; Quillent *et al.,* 1996). In contrast to HIV, the contribution of the *pol* region in the particle production by HTLV-I and HTLV-II has not yet been investigated. In the present study, we produced mature HTLV-II viral particles and examined the involvement of the *pol* region in particle formation using a recombinant baculovirus system.

RESULTS

Analysis of virus particle production using the complete *gag-pro-pol* gene product

In HIV-1, the expression of both Gag and Pol proteins with the use of a recombinant virus results in the processing and production of mature particles with

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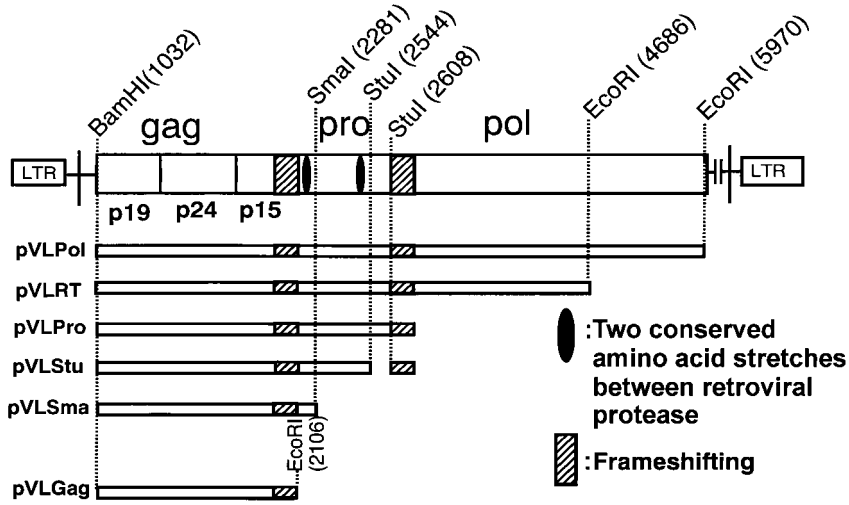

FIG. 1. Schematic map of six mutant proviruses. The HTLV-II clone used for this study was pBR322, 3′ HTLV-IIa (Mo-T)-proviral sequence (Shimotohno *et al.,* 1985). For each proviral construct, the boundaries of the deletions are presented together with the corresponding restriction sites, indicated by broken lines. Striped areas indicate overlapping regions between two coding regions. Filled ovals indicate two conserved amino acid stretches between retroviral proteases. The transfer vectors were designated as pVLPol, pVLRT, pVLPro, pVLStu, pVLSma, and pVLGag. Using these transfer vectors, the recombinant baculoviruses were prepared and designated as vAcPol, vAcRT, vAcPro, vAcStu, vAcSma, and vAcGag, respectively.

electron-dense cores similar to wild-type HIV virions (Ross *et al.,* 1991). To further evaluate the production of mature HTLV-II-like particles in insect cells, we prepared recombinant baculoviruses encoding the Gag-Pro-Pol polyproteins. The expression vector pVLPol, which contains the entire *gag-pro-pol* coding region, is shown in Fig. 1. To investigate the effects of mutations in the HTLV-II *pol* coding region, as described later, we generated a series of transfer vectors, including *pol* region deletion mutants (pVLRT and pVLPro), pVLStu, pVLSma, and pVLGag (Fig. 1).

Insect cells were infected with the recombinant baculovirus vAcPol and analyzed by immunoblotting with rabbit anti-serum p24. Protein p24 was detected in both the infected cells (Fig. 2A, lane 1) and the supernatant (Fig. 2B, lane 1), like HTLV-IIa chronically infected Mo-T cells (Fig. 2A, lane 2) and its supernatant (Fig. 2B, lane 2). These results suggest that mature particles are produced by vAcPol. A 24-kDa protein was also detected using HTLV-II patient serum (Fig. 2C, lanes 3 and 4), as it was using rabbit anti-serum p24 (Fig. 2C, lane 2). As reported in other retroviruses, phosphorylation of the p24 in mammalian cells was seen as a doublet of bands. The 24-kDa protein was not detected in uninfected insect cells (Fig. 2C, lane 1).

Effects of mutation of the HTLV-II *pol* gene on production of p24 capsid protein

The effects of a frameshift mutation in the *pol* gene on particle morphology have been described for HIV-1. Particles that possess protease but not reverse transcriptase (RT) or integrase (IN) activity have a typical immature morphology lacking the electron-dense centers, and the production of p24 is reduced in immunoblotting (Quillent *et al.,* 1996; Ross *et al.,* 1991; Shioda and Shibuta, 1990). Premature termination codons in the HIV-1 *pol* gene, particularly involving the IN-coding region, can also cause marked reductions in viral particle yield (Bukovsky and Göttlinger, 1996). These data suggest an important role of the *pol* region in viral assembly, budding, and maturation.

Regarding HTLV-II, pVLRT and pVLPro mutants, which lack IN (Balakrishnan *et al.,* 1996) or the whole Pol protein, did not seem to affect the yield of p24 protein (CA) (Figs. 3A, lanes 5 and 6, and 3B, lanes 4 and 5) in the baculovirus system. We had the same results from further deletion extending into the protease region (*pro*) of pVLStu (Figs. 3A, lane 4, and 3B, lane 3). However, we could not deny that p24 contained nonspecifically and partially processed proteins by viral protease (Pro). As expected, when the one of the protease active sites was removed in pVLSma, CA was no longer observed (Figs. 3A, lane 3, and 3B, lane 2).

To determine whether expression of HTLV-II Gag protein is sufficient for viral particle production, we produced the $Pr53^{gag}$ through the use of the recombinant baculovirus encoding $Pr53^{gag}$, vAcGag. As shown in Fig. 3A (lane 2), a band at 53 kDa was detected in the extract of high 5 (H5) cells infected with vAcGag. This 53-kDa protein comigrated with the $Pr53^{gag}$ produced in the HTLV-IIa-infected Mo-T cell line (Fig. 3A, lane 8). We also observed several additional bands of lower molecular mass. However, no molecular species comigrating with authentic p19, p24, or p15polypeptides (Fig. 3A, lane 2) were detected. SDS–PAGE analysis of infected H5 cell medium also showed a major band migrating at 53 kDa in cells infected with vAcGag (Fig. 3B, lane 1). The same results were ob-

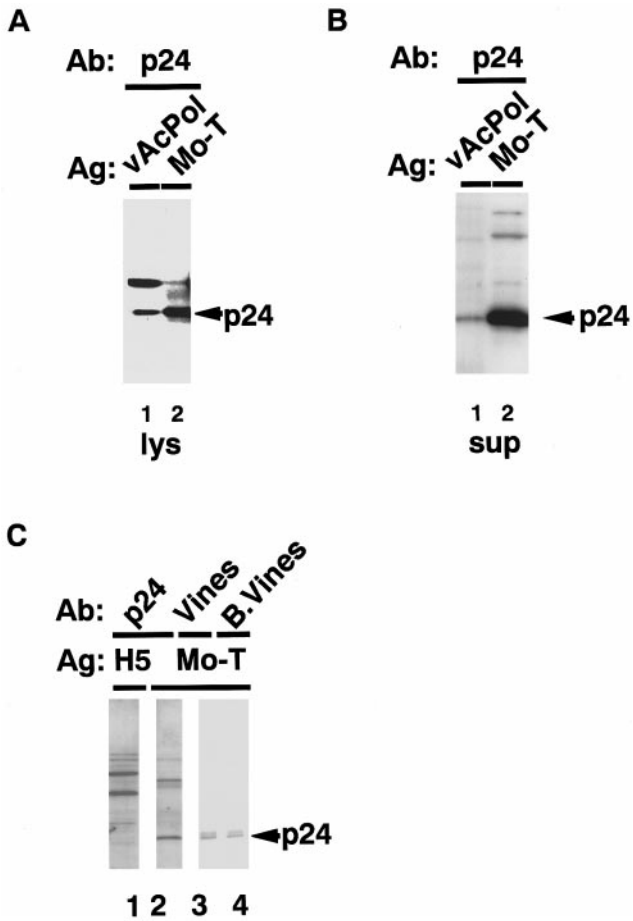

FIG. 2. Immunoblotting of the HTLV-II Gag capsid protein p24 (CA) in full *gag-pro-pol* genome expression. Insect cells infected with vAcPol. (A) The HTLV-II-specific proteolysis of Gag precursor produced p24 in H5 cells infected with recombinant vAcPol baculovirus (lane 1) and in the HTLV-IIa chronically infected cells (Mo-T) (lane 2). They were examined with the use of 1:2000 diluted rabbit anti-serum p24. (B) In both culture mediums, the production of capsid protein p24 was examined. (C) Cell extracts of uninfected insect cells (H5) and Mo-T were immunoblotted by rabbit anti-serum p24 (lanes 1 and 2). Cell extracts of Mo-T were examined by immunoblotting with HTLV-II patient sera Vines (lane 3) and B. Vines (lane 4). Ab indicates antibody; Ag, antigen.

tained using the recombinant vAcSma (Figs. 3A, lane 3, and 3B, lane 2), probably because the protease region is also inactivated in this construct. Although efficiency of $Pr53^{gag}$ production by vAcGag was not so much as that produced by vAcSma, the amount of $Pr53^{gag}$ in supernatant was parallel to that in infected cells. From these results, efficiency of the particle release from infected cells may not differ between vAcGag and vAcSma.

In both cell extracts and the culture medium, the amount of $Pr53^{gag}$ was much smaller than that of p24 (Figs. 3A, lanes 4–8, and 3B, lanes 3–7). These results also support the view that HTLV-II protease protein (Pro) is clearly active not only in released particles but also in infected cells.

Characterization of particles produced by recombinant baculoviruses

Viral particle formation by these mutant Gag-Pro-Pol proteins was confirmed through sucrose density gradient analysis. Fractions were separated on an SDS–polyacrylamide gel and immunoblotted using HTLV-II patient serum. In all cases, antigen peaks of p24 were detected at a sedimentation density of $\rho = 1.15$ (g/ml) (Figs. 4, A–E). To characterize further the recombinant $Pr53^{gag}$ secreted into the culture medium, we performed sucrose density gradient analysis (Figs. 5A and 5B). $Pr53^{gag}$ was recovered between the densities of 1.15 and 1.20 g/ml, suggesting that $Pr53^{gag}$ was secreted as viral particles. The wide distribution of HTLV-II Gag proteins across the gradient may be explained by the production of variable amounts of complete and incomplete particles.

Production of HTLV-II-like particles

We confirmed the production of mature HTLV-II-like particles on electron microscopy resembling those present in the Mo-T cell line (Figs. 6A and 6B). The recombinant baculoviruses that contain protease active sites (vAcPol, vAcRT, vAcPro, and vAcStu) produced mature virus-like particles (Figs. 7A, vAcPol; 7B, vAcRT; 7C, vAcPro; and 7D, vAcStu). Morphological examination by electron microscopy confirmed the production of mature viral particles.

We examined the morphology of the viral particles produced from $Pr53^{gag}$ by electron microscopy. The spherical particles were ~100 nm in diameter, the same as particles present in the Mo-T cell line, but they did not contain central cores (Fig. 8A; indicated by * or **). Our results demonstrate that the uncleaved HTLV-II Gag protein precursor assembles into virus-like particles without the expression of any other HTLV-II gene products.

DISCUSSION

In this study, we have shown that a recombinant baculovirus expressing Gag-Pro-Pol polyproteins (vAcPol) could produce mature particles. The Gag-Pro-Pol polyprotein construct (pVLPol) should be capable of producing all of the proteins required for particle maturation. We were able to produce mature particles in insect cells using vAcPol, and the expected electron-dense centers were observed in these particles. The maturation of the particles was confirmed by immunoblotting and electron microscopy. This suggests that HTLV-II protease (Pro) is expressed by the recombinant baculovirus, resulting in the processing of $Pr53^{gag}$. In support of this, the recombinant virus vAcSma, which expresses Gag and Gag with a deleted Pro (Figs. 3A and 3B, indicated by *), did not produce mature particles. This result implies that capsid production is associated with the intracellular expression of viral protease Pro and may not cellular enzymes. However, we cannot completely exclude the influence of

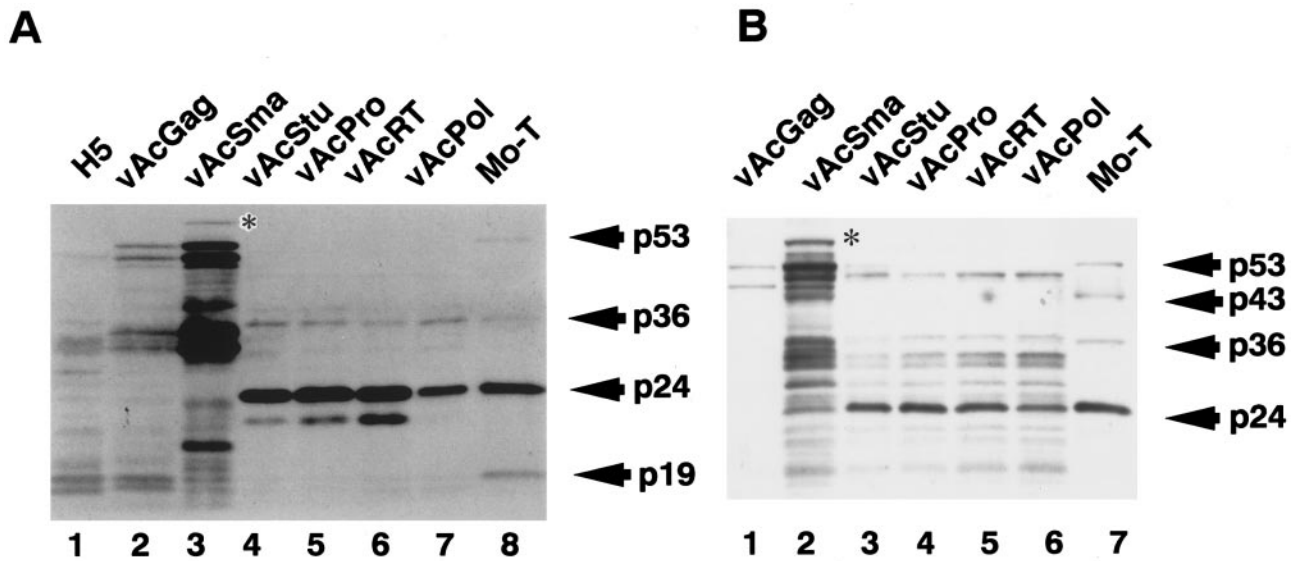

FIG. 3. H5 insect cells were infected with the recombinant baculoviruses; 72 h after infection, cell extracts (A) were prepared in sample buffer, after which the supernatant fractions (B) were pelleted by ultracentrifugation. Viral pellets were separated on SDS–polyacrylamide gel. The transfer membrane was incubated with diluted HTLV-II patient serum (Vines). (A) The 53-kDa proteins observed in lanes 2 and 3 produced in insect cells appeared as the HTLV-II $Pr53^{gag}$ polyprotein precursor. The 24-kDa proteins (p24) were detected in lanes 4–8. (B) Major bands migrating to 53 kDa were also detected in the infected H5 cell supernatants (lanes 1 and 2). These results suggest that $Pr53^{gag}$ is assembled in H5 cells and released to the extracellular space. P24 was also detected (lanes 3–7).

cellular proteases in particle formation at this time. In fact, we did observe other types of immature viral particles with doughnut shapes without electron-dense centers (Fig. 8B→) (Bazarbachi *et al.,* 1994). In addition, it is possible that these could have resulted from processing by cellular proteases. Moreover, this might explain the detection of different proteins with unexpected molecular sizes as seen in Figs. 3A (lanes 2 and 3) and 3B (lanes 1 and 2). This processing by cellular protease were remarkable in insect cells because in mammalian cells, unexpected bands were hardly seen (Figs. 3A, lane 8, and 3B, lane 7). It seems that the baculovirus system could not accurately reproduce the authentic virus situation.

We also analyzed the roles of Pol and Pro in the particle maturation process. To examine whether Pol and Pro proteins are required for particle maturation or production in HTLV-II, we generated deletion mutants of the *pro* and *pol* regions as described in Fig. 1. In HIV-1, when the *gag-pol* ORF is truncated to the *pro* domain to limit Pol expression, some particles lacking the condensed core structures were found (Quillent *et al.,* 1996; Ross *et al.,* 1991). Reduction of particle maturation is thought to be affected by both the size and the position of the deletions in *pol* (Quillent *et al.,* 1996). Furthermore, a marked reduction in particle production is also seen when premature termination codons are introduced into the HIV-1 IN-coding region (Bukovsky and Göttlinger, 1996). In HTLV-II, our experiments demonstrated that truncation of the *pol* region in HTLV-II did not affect particle maturation and production as clearly as shown in HIV-1 (Quillent *et al.,* 1996; Ross *et al.,* 1991; Shioda and Shibuta, 1990). Viral particles with the expected buoyant density and containing p24 were found to be produced by all of the *pol* mutants. With regard to Pro, only when one of the two conserved amino acid stretches that were part of the proteolytic active site were truncated (vAcSma), immature particles were produced. On the other hand, the recombinant baculovirus expressing a deletion in the 3′ end of the Pro domain (vAcStu) hardly affected particle maturation and production. This is partly because the 3′ region of the putative HTLV *pro* region, extending from the second conserved amino acid stretch to the carboxyl-terminal residue, is longer than the analogous region of other retrovirus *pro* regions (Nam and Hatanaka, 1986). Despite the high level of amino acid homology found in this region among members of the HTLV family, the biological function of this region remains unclear. Thus truncation of HTLV-II Pro or Pol may not affect particle formation as clearly as HIV-1 (Quillent *et al.,* 1996; Ross *et al.,* 1991; Shioda and Shibuta, 1990), and it appears that the system of particle formation is different between lentiviruses and oncoviruses.

One difference in the particle formation between HIV-1 and HTLV-II is that the HTLV-II Gag-Pro-Pol precursor protein ($Pr160^{gag\text{-}pro\text{-}pol}$) is produced in much lesser quantities compared with HIV-1 (1:100) (Hatanaka, 1991). The reduced production of $Pr160^{gag\text{-}pro\text{-}pol}$ may be related to the fact that the frameshift efficiency in translating Gag-Pro-Pol of HTLV-II is very low (0.05%) (Hatanaka, 1991). To produce $Pr160^{gag\text{-}pro\text{-}pol}$, two frameshift events are required. In contrast, HIV-1 requires only a single frameshift to produce Gag-Pol precursor proteins.

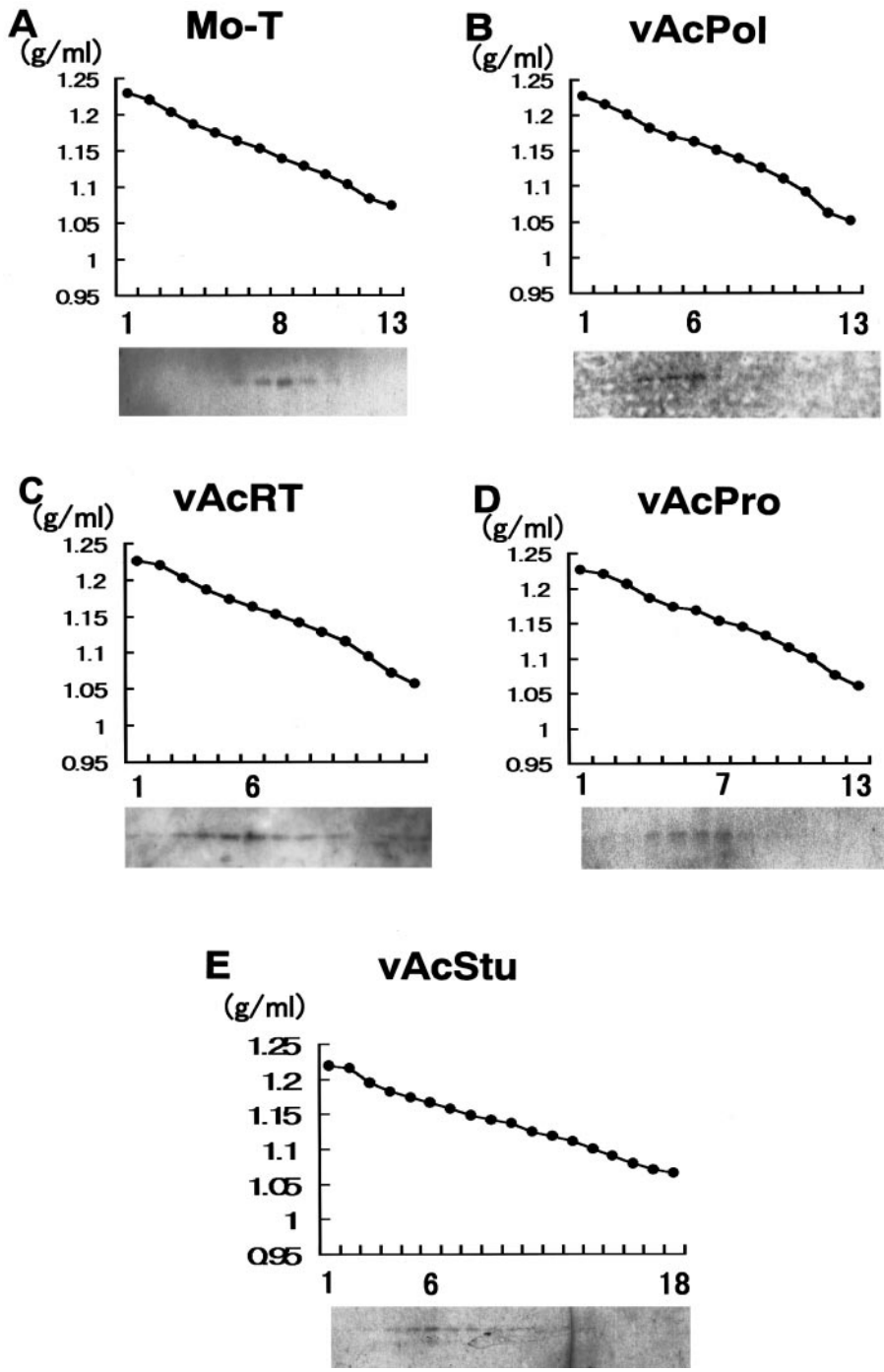

FIG. 4. Characterization of particles produced by Mo-T cell (A) line and insect cells infected with recombinant baculoviruses (B–E). The density of each fraction is indicated on the left. Immunoblot of sucrose density gradient fractions are given below. To further characterize the viral antigens in the culture medium, pelleted material from clarified culture medium was fractionated on a linear 20–60% sucrose density gradient for 16 h at 35,000 rpm. Fractions were collected and separated on SDS–polyacrylamide gel. The transfer membrane was incubated with diluted HTLV-II patient serum (B. Vines). Antigen (p24) peaks were detected (A–E), sedimenting at ~1.15 g/ml in HTLV-II particles from Mo-T cells (A) and all kinds of HTLV-II-like particles from recombinant baculoviruses vAcPol (B), vAcRT (C), vAcPro (D), and vAcStu (E). We described the number at the peak of each band.

Other differences between HTLV-II and HIV-1 are that the Gag-Pro polyproteins exist independently of Gag-Pro-Pol polyproteins. Hence, mutation of the *pol* region, which affects RT and IN, does not influence protease activity. The Gag-Pro polyproteins should be sufficient to form morphologically mature viral particles (Fig. 6C). Second, HTLV-II Pro appeared to be very active in infected cells before or during the budding process, suggesting that HTLV-II Gag-Pro proteins are autoprocessed to form mature particles by themselves. Therefore, HTLV Pro is thought to be markedly active, and the precursor proteins are rapidly cleaved in infected cells. This would

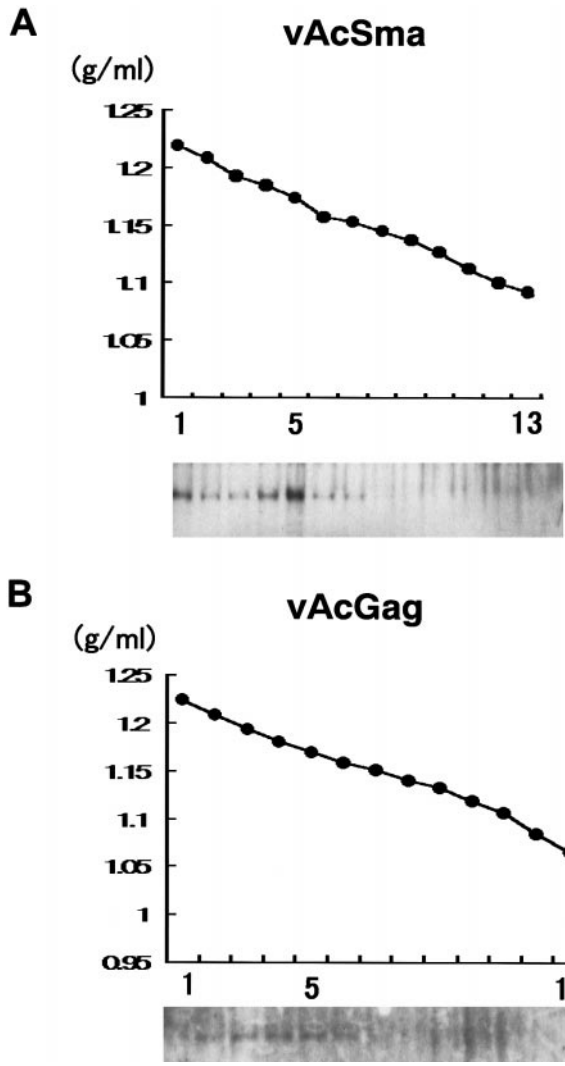

FIG. 5. Antigen peak of Pr53^{gag} was also detected at ~1.15 g/ml in vAcSma (A) and vAcGag (B). Antigen peaks across the gradient were observed.

explain the observation that the precursor protein Pr53^{gag}, which is indispensable for budding, was barely detected in the infected cells by immunoblotting (Fig. 3A, lanes 4–8). In contrast, HIV-1 Pro is usually active after budding, and HIV-1 Pr55^{gag} is readily detectable in infected cells (Bukovsky and Göttlinger, 1996). The difference in activities between HTLV-II Pro and HIV-1 Pro may depend on different homologies and cleavage sites of these proteases. Thus active HTLV-II Pro in infected cells would also explain why little or no Pr160$^{gag\text{-}pro\text{-}pol}$ or truncated Pr160$^{gag\text{-}pro\text{-}pol}$ products were detected.

Although some reports suggest that HIV-1 virion assembly could be affected by mutation in the *pol* region (Engelman *et al.,* 1997; Shin *et al.,* 1994), others do not always show a requirement for complete *pol* region. Point mutations in IN affect assembly, but deletion of the RT and IN barely affected the particle production (Bukovsky and Göttlinger, 1996). We speculate that these discrepancies probably are attributed to the effect of the mutations on the dimer formation induced by the IN, and the length of the *pol* region was not always proportioned to the suppression of assembly.

Last, we have shown that HTLV-II Pr53^{gag} is produced in insect cells (H5 cells) using a recombinant baculovirus vAcGag. In the insect system, HTLV-II Pr53^{gag} assembles at the cell plasma membrane for budding and is released into the culture medium as particles of ~100 nm in diameter (Fig. 8A, indicated by **). Electron microscopy of thin sections of infected cells showed copious budding structures of the particles (Fig. 8B, indicated by *), which displayed a translucent center and a thick, peripheral electron-dense ring surrounded by an outer lipid bilayer membrane of host-cellular origin that was ac-

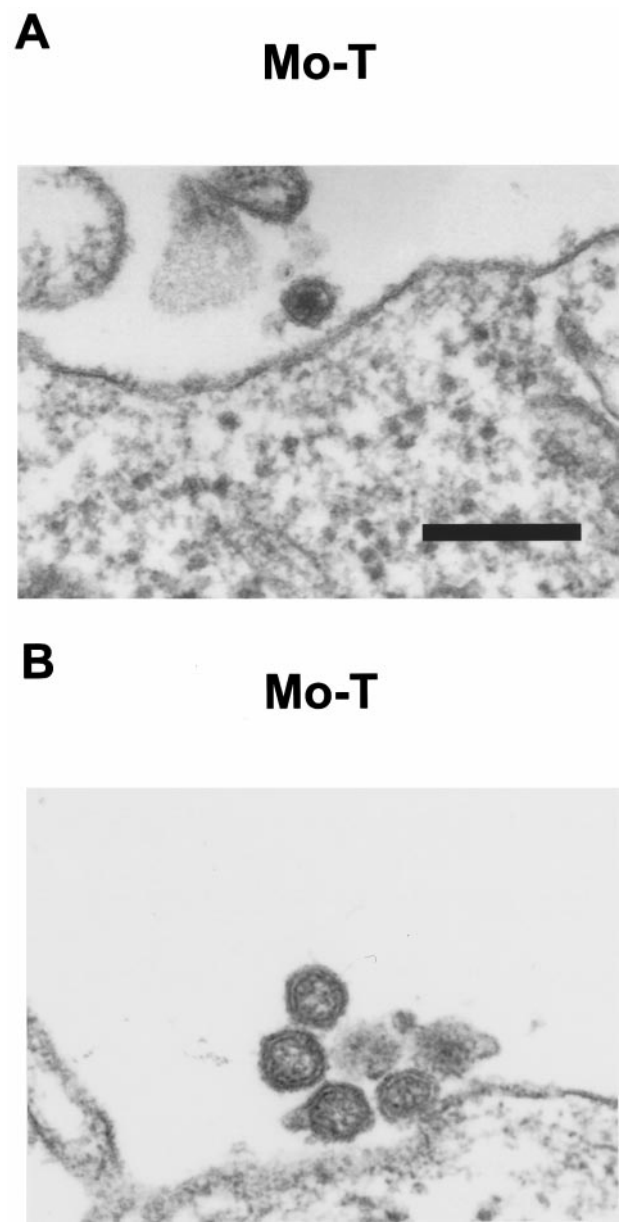

FIG. 6. Electron microscopy of Mo-T cells (A and B). Morphologically, we confirmed mature (A) and immature (B) HTLV-II particles produced by Mo-T cell line. Magnification bar = 0.5 μm.

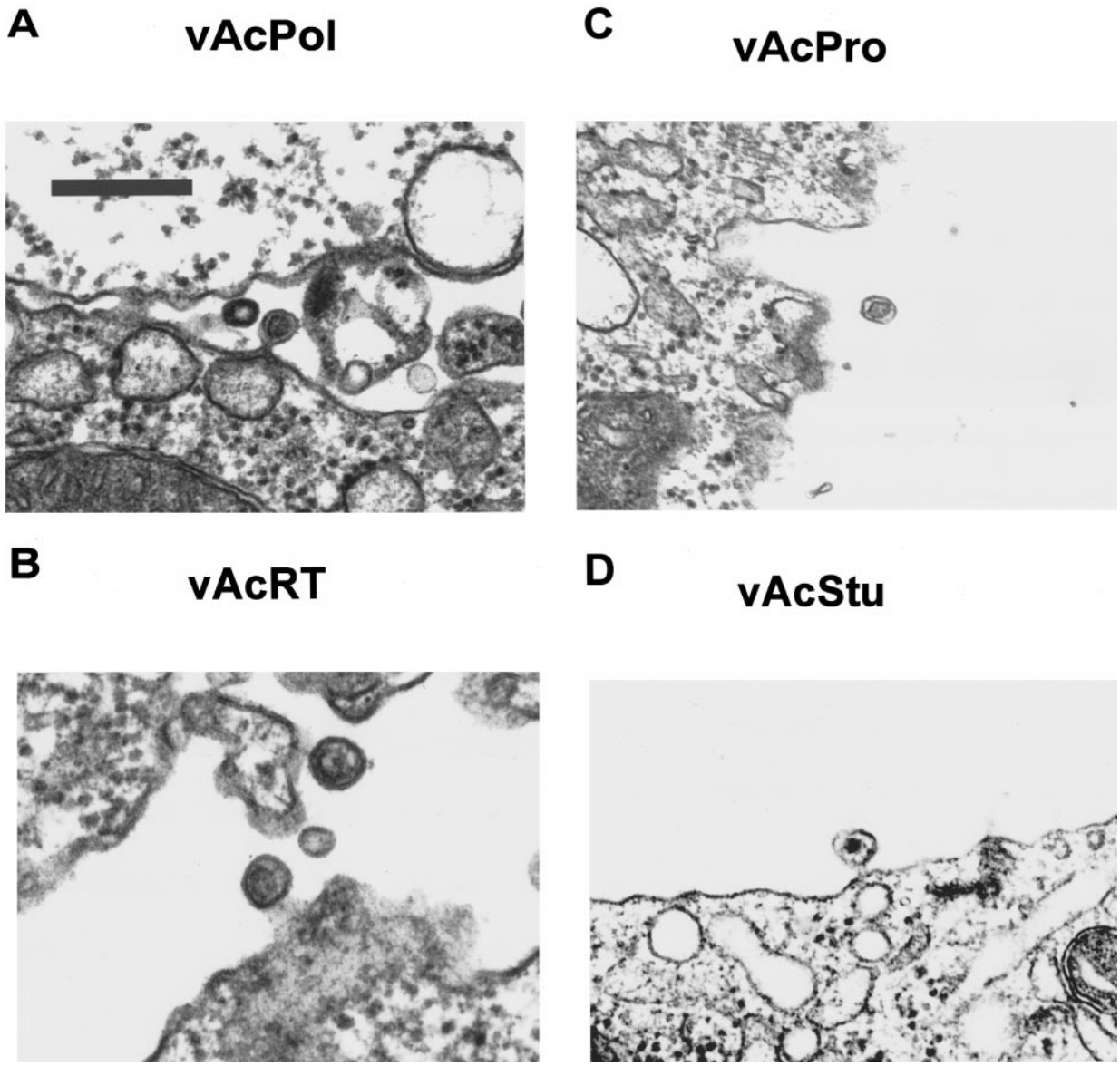

FIG. 7. Electron microscopy of H5 cells infected with recombinant baculoviruses. Twenty-four hours after infection, H5 cells were removed from culture dishes and were fixed. H5 cells were infected with recombinant baculoviruses vAcPol (A), vAcRT (B), vAcPro (C), and vAcStu (D). Morphologically, we confirmed mature HTLV-II-like particles produced by various recombinant baculovirus-infected insect cells (A–D). Magnification bar = 0.5 μm.

quired during the budding process. In summary, the process of precursor Gag particle formation in these H5 cells closely resembles the budding of immature HIV-1 retrovirions (Carriere *et al.,* 1995, Gelderblom *et al.,* 1987; Gheysen *et al.,* 1989; Katsumoto *et al.,* 1987) with respect to size, physical shape, and typical ring structure. These immature retrovirions are also produced by Gag of HIV-2 (Luo *et al.,* 1994) and bovine immunodeficiency-like virus (Rasmussen *et al.,* 1990). Studies with moloney murine leukemia virus, which is also a type C virus, have indicated that core structures are first formed by the association of uncleaved Gag precursor monomers (Lu *et al.,* 1979), and proteolytic processing occurs only during or after budding (Crawford and Goff, 1985). The HTLV-II-like particles produced by the recombinant baculovirus vAc-Gag showed exclusively immature particles without electron-dense cores composed of capsid proteins.

MATERIALS AND METHODS

General DNA methods

Restriction endonucleases and DNA-modifying enzymes were used as specified by the manufacturers. The enzymes used were *Bam*HI, *Eco*RI, *Eco*RV, *Sma*I, *Stu*I, *Xba*I, alkaline phosphatase, and T4 DNA ligase and were obtained from Boehringer Mannheim (Indianapolis, IN). All molecular biological manipulations were carried out by standard methods (Nam *et al.,* 1993). All DNA transformations were performed with competent *E. coli* DH 5α cells (Toyobo).

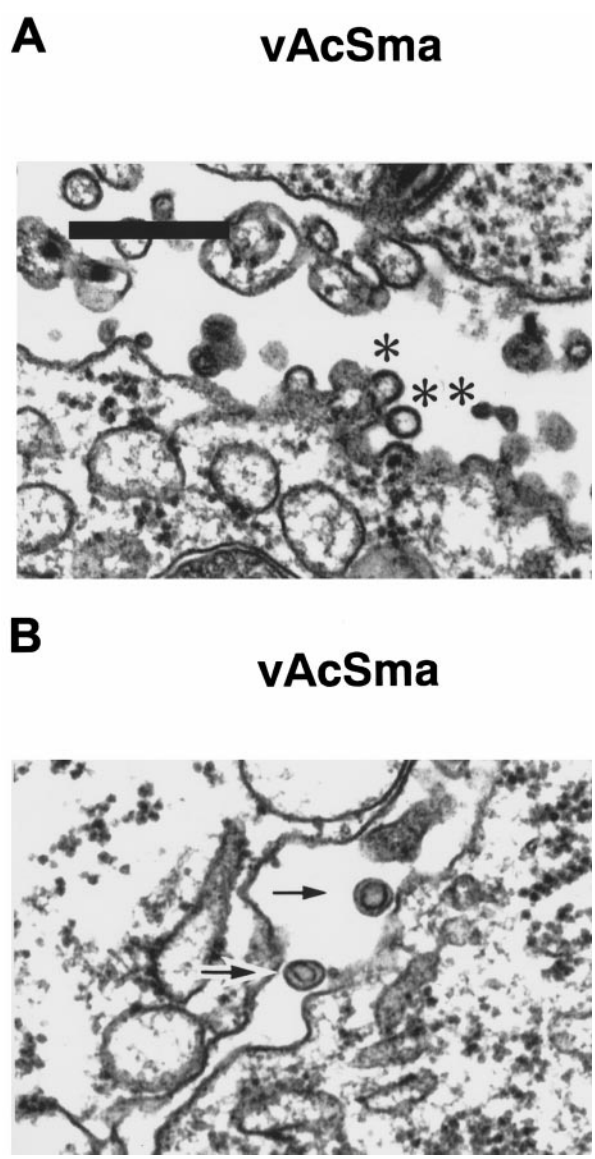

FIG. 8. Electron microscopy of H5 cells infected with recombinant baculoviruses vAcSma (A and B). Electron micrograph of insect cells infected with the vAcSma recombinant baculovirus, electron-dense areas protrude from the plasma membrane of vAcSma-infected H5 cells, consistent with early budding (*) and late budding (**). Doughnut-shaped particles are also seen (B→). Magnification bar = 0.5 μm.

Construction of recombinant baculoviruses expressing mutant forms of the *gag, pro,* and *pol* gene regions

Sequence information used for cloning is based on that reported by Shimotohno *et al.* (1985) and involved the use of the 3.7-kb *Eco*RV–*Eco*RI fragment of clone pBR322, which contains the 5′ half of the HTLV-IIa provirus. The relative map positions of all of the constructs are shown in Fig. 1. The purified DNA fragment was ligated with *Eco*RV-*Eco*RI-digested pVLPro, pVLRT′. The sequence of the *gag-pro* gene was generated by PCR using 5′-CTCCATTCTGGATCCATGGGACAAATC-3′ for the forward primer and 5′-TGGAGACCAGAATTCTAAAGGCCTGGA-3′ for the reverse primer. The underlined sequences are the *Bam*HI and *Eco*RI sites, respectively. The 1.6-kb *gag-pro* fragment generated by PCR was inserted into the *Bam*HI and *Eco*RI sites of the pVL1392 (PharMingen, San Diego, CA) and named pVLPro. The 3′ portion of the *pol* and the 5′ portion of the *env* gene at positions 5089–5960 in clone pBR322 were introduced into the *Eco*RI–BamHI sites of the pBluescript IIKS$^{(+)}$ (Stratagene) by using PCR. Reverse and forward oligonucleotide primers were 5′-CCCCCGCCCGGATCCCGTGGCGATT CCTGA-3′ and 5′-GGTTGGGATGAATTCGCGGGCAGGGCA-3′, respectively. The plasmid constructed was named pBS-II3′. The underlined sequences are the *Bam*HI and *Eco*RI sites. The *Xba*I–*Bam*HI fragment (nucleotides 3685–5089) in the pBR322 was inserted into the *Bam*HI–*Xba*I site of pBS-II3′, which was called pBS-II3′-II5′. pBS-II3′-II5′ was digested with *Eco*RI. The 1.3-kb *Eco*RI fragment and the 0.3-kb *Eco*RV fragment from pVLPro were ligated with dephosphorylated *Eco*RI and the *Eco*RV site of pVLRT′, respectively. The constructed plasmid was designated pVLPol. To construct pVLRT, pVLPol was digested with *Eco*RI. The pVLRT is a deletion mutant of the IN region (Balakrishnan *et al.,* 1996). To construct pVLStu and pVLSma, pVLPro was digested with *Stu*I and *Sma*I, respectively. PVLGag was generated by PCR using 5′-CTCCATTCTGGATCCATGGGACAAATC-3′ and 5′-CGGGATGGGGAATTCTTAGATCTCCCC-3′, as forward and reverse primers, respectively. The underlined sequences are the *Bam*HI (forward) and *Eco*RI (reverse) sites. The 1.3-kb fragment generated by PCR was inserted into *Bam*HI and *Eco*RI sites of pVL1392.

Cells and viruses

The H5 insect cells (InVitrogen) originating from the ovarian cells of the cabbage looper *Trichoplusia ni* were propagated at 27°C in TC 100 medium containing 10% FBS. Baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) and recombinant baculovirus were grown in H5 cells. Recombinant baculoviruses were generated by cotransfecting H5 cells with wild-type baculovirus AcNPV (BaculoGold; PharMingen) viral DNA (0.1 μg) together with 2 μg of the transfer vector (pVLPol, pVLRT, pVLPro, pVLStu, pVLSma, and pVLGag) as described previously (Smith *et al.,* 1985). Culture supernatants were harvested 3 days after transfection. H5 cells were infected for 1 h with recombinant viruses in culture supernatants. The infected cells were seeded in 25-cm^2 spinner flasks (Nunclon) and incubated at 27°C. The recombinant baculoviruses were designed vAcPol, vAcRT, vAcPro, vAcStu, vAcSma, and vAcGag.

SDS–PAGE and polyclonal antibody production

SDS–12% PAGE was used to analyze the virus proteins. Polyclonal antibodies to the HTLV-II p24 were generated by injecting the highly purified protein into rabbits.

Incomplete Freund's and complete Freund's adjuvants (DIFCO, Detroit, MI) were used as an adjuvant in a 1:1 ratio with the antigen (used according to the instructions of the manufacturer). One milliliter of the purified p24 protein was combined with 1.0 ml of the complete Freund's and injected into the footpad. Then 1.5 ml of the same p24 was combined with 1.5 ml of incomplete Freund's and given as 1.5 ml of subcutaneous injections every 7 days for three times. After 10 days, 20 ml of the rabbit blood was collected and centrifuged, and the sera were frozen at −20°C. Reactivity of the antibody was determined by Western blot analysis as described previously (Tanese *et al.,* 1986).

Western blot analysis of viral proteins

Virus lysates were prepared from infected H5 cells. Cell-free supernatants harvested 72 h after infection were centrifuged through 20% sucrose cushions in PBS by ultracentrifugation at 26,000 rpm (SW 41; Beckman) for 2 h. Viral pellets were resuspended in PBS and proteins were subjected to SDS–12% PAGE. After semidry transfer to nitrocellulose membranes (Immobilon, Millipore), the proteins were exposed to a mixture of p24 rabbit polyclonal antibodies (1:2000 diluted) or serum (1:2000 diluted) from individuals infected with HTLV-II. Blots were developed by enhanced chemiluminescence (SuperSignal ULTRA; Pierce) after incubation with horseradish peroxidase-conjugated anti-rabbit (Souther Biotechnology Associates) or anti-human antibody (Tago).

Sucrose density gradient analysis

Pelleted material from clarified culture medium, prepared as described above, was fractionated on a linear 20–60% sucrose density gradient for 16 h at 35,000 rpm (SW55Ti; Beckman). Fractions (425 μl) were collected and 10 μl were subjected to immunoblotting as described previously. Densitometry was measured using a refractometer. The relative antigen (Ag: $Pr53^{gag}$, p24) concentrations were determined by Western blot.

Electron microscopy of wild-type and mutant viral particles

Twenty-four hours after infection, H5 cells were removed from culture dishes by pipetting, pelleted by low-speed centrifugation, fixed in 2.5% glutaraldeyde/5.4% sucrose cacodylate, and then postfixed. After ethanol dehydration and embedding in epoxy resin, thin sections were made, which were further stained with uranyl acetate and lead citrate and examined on a JEM 1200 EX II Joel (Tokyo, Japan) electron microscope.

ACKNOWLEDGMENTS

This investigation was supported by the Japanese Foundation for AIDS Prevention. We are grateful to M. Matsuda (Department of Pathology, Research Institute, International Medical Center of Japan) and K. Tokunaga (Unite de Biologie des Retrovirus, Departement de Virologie, Institut Pasteur) for helpful discussions and comments.

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